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Phylogeography and genetic diversity of *Adineta beysunae* and *Rotaria sordida* species complexes (Rotifera, Bdelloidea): a combined pattern of widespread and localized distribution driven by climate factors

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Abstract

Understanding the driving factors of the biogeography of microscopic animals is crucial for global biodiversity research. However, their geographical distribution patterns remain poorly resolved, with ongoing debate regarding the primary roles of climate, geography, and human activities. In this study, we investigated the genetic diversity and lineage distribution of two understudied bdelloid rotifer species complexes—*Adineta beysunae* Örstan, 2018 and *Rotaria sordida* (Western, 1893)—across 15 and 35 geographic regions (primarily in China and Europe), respectively. Phylogenetic analyses based on mitochondrial COI markers identified 10 distinct clades in *A. beysunae* and 58 in *R. sordida*, revealing high cryptic diversity. Analysis of Molecular Variance (AMOVA) indicated that genetic variation in both species occurred predominantly within populations (*A. beysunae*: 75.26%; *R. sordida*: 75.55%) rather than among populations. These results demonstrate a heterogeneous phylogeographic structure characterized by widespread lineages and fragmented endemic clades (e.g., island-restricted lineages). Hierarchical partitioning analysis identified climatic factors—particularly seasonal temperature variation, annual precipitation, and mean wind speed—as the dominant drivers of phylogeographic patterns in Chinese populations, surpassing the effects of spatial variables, including longitude and elevation. Collectively, this study uncovers high cryptic diversity in *A. beysunae* and *R. sordida*, quantifies the relative contributions of key driving factors, and supplements existing knowledge of microfaunal biogeographic patterns, providing foundational data to elucidate global mechanisms of micro-metazoan diversification and adaptive evolution.

Key Words

Bdelloid rotifer, biogeography, COI, community ecology, invertebrate infraspecific diversity

Introduction

Biodiversity loss driven by global climate change and human activities has emerged as a critical ecological challenge (Otto 2018; Mahecha et al. 2022). As key components of ecosystem functioning, microscopic invertebrates (<2 mm) remain understudied compared to larger animals, particularly regarding their diversity distribution patterns, and consequently hold a lower priority in biodiversity conservation (Martínez et al. 2025; Wu et al. 2025). Due to their small body size, limited morphological variation, and capacity for multi-vector dispersal, microscopic animals were once hypothesized to lack geographical barriers and exhibit cosmopolitan distributions. However, this view has been challenged by subsequent discoveries of restricted distributions in various groups, including ciliates (Dunthorn et al. 2012), tardigrades (Guidetti et al. 2019), and rotifers (Martiny et al. 2006; Fontaneto 2011), with numerous endemic species reported from geographically isolated and unique environments such as Antarctica and Australia (Segers and Shiel 2008; Iakovenko et al. 2015). Advances in molecular biology and genomic sequencing

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have further revealed multiple cryptic lineages within microscopic animals, for example, among monogonont rotifers (Deng et al. 2022a; Liang et al. 2022), nematodes (Poureskandarian et al. 2023), tardigrades (Surmacz et al. 2025), and protists (De Luca et al. 2021), that often exhibit restricted distribution patterns akin to those of larger animals (Fontaneto et al. 2008; Deng et al. 2022a). Nevertheless, accurate assessment of diversity distribution in microscopic animals faces several major obstacles: a large proportion of undescribed species, markedly fewer data from regions outside Europe, and a lack of comparable datasets (Martínez et al. 2025). This is especially true for patterns of genetic diversity, for which available data—and the geographic coverage of these data—remain particularly scarce (Martínez et al. 2025). Such data, however, are essential for uncovering the macroecological mechanisms that shape the geographical distribution of microscopic animals (Martínez et al. 2025).

Further research has sought to disentangle the driving factors shaping the biogeographical distributions of microscopic invertebrates. Spatial and environmental factors dominate these discussions (Burgazzi et al. 2020), with many species exhibiting distance–decay relationships, also known as isolation by distance (IBD). For example, spatial autocorrelation has been reported for bdelloid rotifer communities at distances between 54 and 133 meters, beyond which communities become highly dissimilar (Robeson et al. 2011). This distance effect is also reflected in genetic differentiation levels, where population dissimilarity increases with geographical distance (Liang et al. 2022). Beyond spatial factors, some microfauna, including cryptic species nested within morphological taxa, display distinct ecological niches (Gabaldón et al. 2017; Walczyńska et al. 2024). Their population distributions and successional dynamics are dictated by specific environmental variables (Burgazzi et al. 2020). Moreover, within the Anthropocene context, human activities further exert strong selective pressures on animal assemblages (Yu et al. 2025). It is important to note that these factors do not operate in isolation when influencing the geographical distribution of microscopic animals; instead, they may exhibit coupled effects (Holman et al. 2021). An integrated study of nearshore metazoan, protistan, and bacterial communities revealed consistent response patterns jointly influenced by environmental conditions, geographic location, and anthropogenic factors (Holman et al. 2021). However, the relative contributions and interactions of these three driving factors remain unresolved.

Bdelloid rotifers (Rotifera, Bdelloidea) encompass over 500 documented species worldwide, with body lengths mostly ranging between 150 and 700 μm (Ricci and Melone 2000; Segers 2007; Nowell et al. 2018; Wang et al. 2025). They exhibit unique biological traits, including obligate parthenogenesis, anhydrobiosis (the capacity to enter dormancy in response to environmental stresses such as desiccation), and horizontal gene transfer (Ricci and Fontaneto 2009; Ricci 2017). Many bdelloids, unlike most monogononts, are well adapted to habitats that dry out frequently and in an unpredictable manner, because they can immediately switch to anhydrobiosis as adult individuals instead of producing resting eggs. This life history strategy underlines their broad ecological adaptability to extreme conditions, including subzero temperatures (Shmakova et al. 2021; Raymond 2025), high UV radiation (Wilson et al. 2024), and desiccation (Wilson et al. 2024). Notably, these animals can remain viable for at least 24,000 years in frozen environments (Shmakova et al. 2021). The perceived distribution range of bdelloid rotifers narrows progressively with increasing taxonomic resolution, from the entire subclass down to species and further to lineage levels (Fontaneto et al. 2008). For example, while morphospecies such as *A. vaga* and *R. rotatoria* are considered cosmopolitan, they harbor diverse internal lineages, including both lineages with restricted geographical distributions and those showing evidence of long-distance dispersal (Fontaneto et al. 2008; Zhu et al. 2025). Similarly, geographically restricted lineages have been detected in monogonont rotifers (Deng et al. 2022a) and cladocerans (Ma et al. 2020). This pattern is exemplified by the discovery of numerous endemic lineages of both monogonont rotifers (Deng et al. 2022a) and bdelloid rotifers (Zhu et al. 2025) on the Qinghai–Tibet Plateau, a region characterized by unique climatic and environmental conditions. Bdelloid rotifers are reported to harbor higher cryptic diversity compared to monogononts (Fontaneto et al. 2009). This demonstrates that, despite their obligate asexuality, bdelloids can undergo population isolation and divergent selection leading to speciation, much like their sexual counterparts (Fontaneto et al. 2007). It has been suggested that sympatric speciation might even be accelerated in the absence of sex (Fontaneto et al. 2009). Furthermore, their capacity for horizontal gene transfer from environmental bacteria can fuel rapid adaptive evolution (Raymond 2025). A notable example is the presence of a bacterial-derived ice-binding protein gene in the genome of bdelloid rotifers from Antarctic algal mats, which contributes to their exceptional freeze–thaw tolerance (Raymond 2025). Combined with high dispersal potential via dormant propagules, short generation times, and rapid population growth in suitable habitats (Ricci 2017), these traits may result in phylogeographic patterns for bdelloids that differ significantly from those of sexual or mixed-reproduction microfauna (Fontaneto et al. 2009). Despite being a flagship group for asexual microscopic animals, the phylogeographic patterns of bdelloid rotifers and the mechanisms shaping them remain poorly understood.

Despite growing global research on the genetic differentiation of bdelloid rotifers and an increasing amount of molecular marker and genomic data, these data remain largely concentrated in regions such as Europe and Antarctica (Fontaneto et al. 2019; Cakil et al. 2021). In East Asia, particularly in China, genetic diversity data for bdelloids are still limited. Published phylogenetic analyses cover only approximately 12 species (Wang et al. 2021; Wang et al. 2023b; Zhu et al. 2025). The most comprehensively studied species in the region is *R. rotatoria*,

with a dataset comprising 360 COI and 1080 ITS sequences from 360 individuals across 18 Chinese localities (Zhu et al. 2025). This is followed by *A. beysunae* and *R. sordida*. However, prior to this study, COI sequence data for these two species were primarily available from a single location (Changde), which precluded large-scale phylogeographic analysis (Wang et al. 2023b). For all other bdelloid species recorded in China, the available COI sequences total fewer than 35 (Wang et al. 2021). This research effort is substantially lower than that devoted to monogonont rotifers in China (Xiang et al. 2011; Deng et al. 2022a; Yang et al. 2022). Consequently, the geographic distribution patterns of cryptic species within bdelloid rotifers remain poorly resolved.

China is one of the countries with high biodiversity in the world, containing four out of the 36 recognized global biodiversity hotspots (Mi et al. 2021). Its territory encompasses heterogeneous landscapes, including the Qinghai–Tibet Plateau, Yunnan–Guizhou Plateau, Xinjiang arid zones, northeastern mountains, eastern plains, and offshore islands, where pronounced geographical barriers and climate gradients likely restrict gene flow, potentially driving distinct cryptic lineage evolution, as evidenced in microinvertebrate studies (Ma et al. 2020; Deng et al. 2022a, 2022b; Zhu et al. 2025). To improve understanding of phylogeographic patterns and their drivers in Chinese bdelloid rotifer populations, and to address the current shortage of genetic data from this region, we selected the widely distributed *Adineta beysunae* and *Rotaria sordida* species complexes. We analyzed their COI marker genetic diversity and phylogeographic structure and evaluated the relative effects of geographical, climatic, and anthropogenic factors as potential drivers of genetic diversity. We hypothesized that these two species complexes exhibit high cryptic diversity and display phylogeographic patterns that combine both widespread lineages and localized distributions represented by geographically restricted lineages. This study provides insights into the microevolutionary mechanisms driving diversification in microscopic organisms, clarifies genetic diversity patterns within understudied bdelloids, and ultimately informs conservation prioritization strategies for cryptic invertebrates through the identification of evolutionarily significant units.

Materials and methods

Sampling and species identification

Between 2017 and 2025, we collected 1306 samples, including moss, leaf litter, soil, and lichens, from geographically distinct sites across China. These limno-terrestrial samples were collected in Kraft seed bags, transported to the laboratory, dried at room temperature, and then stored at −20 °C. For rotifer identification, litter, moss, and lichen samples were first soaked in deionized water in conical flasks for 4–24 hours. The soaked material was then vigorously shaken, and the suspension was passed through a 30-μm mesh sieve to collect rotifers. This soaking–shaking–sieving procedure was repeated three times to extract the majority of rotifers present (Wang et al. 2025). This protocol, modified from Peters et al. (1993), allows efficient recovery of active bdelloid individuals. For rotifers inhabiting soil, extraction was performed using the sugar centrifugation method (Freckman and Virginia 1993). Morphological identification of *A. beysunae* and *R. sordida* s. str. was mainly based on the keys of Donner (1965) and the original description of *A. beysunae* (Örstan 2018). Live individuals of these two species used for DNA taxonomy were obtained from 15 and 26 localities in China, respectively (Suppl. material 1: tables S1, S2). Additionally, all available COI sequences of *A. beysunae* and *R. sordida* were downloaded from GenBank (Suppl. material 1: tables S1–S3).

DNA extraction and sequencing

Live *A. beysunae* specimens were washed three times with sterile distilled water and transferred to 0.2 mL PCR tubes. Genomic DNA was extracted from individual rotifers using the HotSHOT method (Montero-Pau et al. 2008). The mitochondrial COI sequence was amplified using primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTG-3′) and HCO2198 (5′-TAAACTTGAGGGTGACCAAAAAAT-3′) (Folmer et al. 1994). Each 25 μL PCR reaction contained 0.125 μL of Ex Taq polymerase, 2.5 μL of 10× PCR buffer, 2 μL of dNTP mix, 0.5 μL each of forward and reverse primers, 14.375 μL of ddH_2O, and 5 μL of template DNA. Thermal cycling conditions were as follows: initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 8 min. PCR products were purified and subjected to bidirectional Sanger sequencing at TsingKe Biotechnology Co., Ltd. (Beijing, China).

Sequence alignment and phylogenetic analyses

In total, we successfully obtained 145 COI sequences of *A. beysunae* and 194 COI sequences of *R. sordida*, including 62 publicly available sequences downloaded from GenBank that represent populations from other regions worldwide (Suppl. material 1: table S1–S3). All COI sequences were manually verified and assembled using SeqMan (DNASTAR Lasergene v7.1) and then aligned in MEGA 7 (Kumar et al. 2016) using the ClustalW algorithm (Thompson et al. 1994). Haplotype identification and frequency analysis were performed in DnaSP 6 (Rozas et al. 2017), and spatial divergence in haplotype frequencies among geographic populations was evaluated using Arlequin 3.5 (Excoffier and Lischer 2010). A Templeton–Crandall–Singh (TCS) parsimony network (Clement et al. 2000) was reconstructed in PopART 1.7 (Leigh and Bryant 2015). For phylogenetic reconstruction, one representative

sequence per haplotype was aligned with two outgroup taxa (*A. beysunae*: *Bradyscela clauda* EF173178 and EF173179; *R. sordida*: *Dissotrocha aculeata* KM043187 and *Dissotrocha macrostyla* KM043188) in PhyloSuite v1.2.3 (Zhang et al. 2019; Xiang et al. 2023). Bayesian inference was conducted using MrBayes 3.2 (Ronquist et al. 2012), with optimal nucleotide substitution models determined separately for *A. beysunae* and *R. sordida* populations using ModelFinder v2.2.0 (Kalyaanamoorthy et al. 2017) under the Bayesian Information Criterion (BIC). The selected models were HKY+invgamma (I+G, proportion invariable, remaining gamma) for *A. beysunae* and GTR+invgamma (I+G, proportion invariable, remaining gamma) for *R. sordida*. Markov chain Monte Carlo (MCMC) parameters included 50 million generations, sampling every 10,000 generations, and a 25% burn-in. Convergence was confirmed when the average standard deviation of split frequencies (ASDSF) fell below 0.01, the potential scale reduction factor (PSRF) approached 1.0, and the effective sample size (ESS) exceeded 100 for all parameters. The consensus tree was visualized and annotated using the iTOL v7 platform (Letunic and Bork 2021; https://itol.embl.de/), with bootstrap values greater than 0.5 displayed at the nodes.

Biodiversity metrics

The number of putative cryptic species or genetic clades within the *A. beysunae* and *R. sordida* species complexes was estimated using the Automatic Barcode Gap Discovery (ABGD; Puillandre et al. 2012) and Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021) methods. For *R. sordida*, additional validation was performed using the Bayesian Poisson Tree Processes (bPTP; Zhang et al. 2013). The inferred clades were mapped to their respective sampling localities to visualize phylogeographic relationships among distinct rotifer lineages. Haplotype diversity (h) and nucleotide diversity (π) were calculated from COI sequences using DnaSP 6. Tajima's *D* and Fu's *FS* values were computed in Arlequin 3.5. Pairwise FST values between populations were calculated in Arlequin 3.5 with 1,000 permutations to quantify genetic differentiation. Analysis of molecular variance (AMOVA) was conducted to examine hierarchical population structure and the geographic patterns of population subdivision in *A. beysunae* and *R. sordida* populations using Arlequin 3.5.

Analysis of drivers shaping phylogeographic patterns

To identify the drivers shaping the phylogeographic patterns of *A. beysunae* and *R. sordida* species complexes in China and to evaluate their relative contributions, we performed a Mantel test to assess correlations between pairwise FST values (genetic differentiation) and geographic distance matrices for both species. Linear regression models were further applied to quantify the association between genetic divergence and geographic distance across sampling regions. Spatial factors were characterized using longitude, latitude, and elevation data. Climatic variables, including annual mean temperature, temperature seasonality, annual precipitation, precipitation seasonality, and annual mean wind speed, were extracted from the WorldClim database (Fick and Hijmans 2017) for each sampling locality. The intensifying impact of human activities on biodiversity is well documented, with numerous studies confirming the relationship between population and economic growth and the loss of habitats and species (Uchida et al. 2025). Population and economic growth drive changes in land use, habitat fragmentation, and increased pollution, all of which contribute to the loss of genetic diversity (Seto et al. 2011; Leigh et al. 2019). In China, increases in urban population size and Gross Domestic Product (GDP) have driven approximately half of urban land expansion (Seto et al. 2011), accelerating the degradation of natural habitats. However, few studies have extended this relationship to the genetic diversity of microscopic invertebrates. In this study, anthropogenic influence was characterized using regional GDP and population density. Additionally, we considered the potential effect of sampling intensity, defined as the number of valid sequences analyzed per population, on estimates of genetic diversity.

The independent contributions of spatial, climatic, and anthropogenic factors to genetic variation in *A. beysunae* and *R. sordida* populations were quantified using hierarchical partitioning analysis (HP) based on distance-based redundancy analysis (dbRDA), implemented in the R package rdacca.hp (Lai et al. 2022). This approach disentangles the unique explanatory power of each predictor while controlling for multicollinearity. All statistical analyses were conducted in R 4.2.2.

Results

Genetic diversity

A total of 145 *A. beysunae* COI sequences with an aligned length of 407 bp were obtained from 15 regions (Suppl. material 1: table S1). The number of polymorphic (segregating) sites (S) was 146, and the total number of mutations (Eta) was 194. A total of 63 haplotypes were identified from these sequences (Suppl. material 1: fig. S1). Overall haplotype diversity was 0.973, and total nucleotide diversity was 0.05341. The ASAP and ABGD models delineated 10 genetic clades (putative cryptic species), among which Clade 10 contained the largest number of haplotypes (32), followed by Clade 8 (14) and Clade 7 (10). Each of the remaining clades consisted of a single haplotype (Table 1). It should be noted that the bootstrap support value for Clade 10 was relatively low (0.499). This low value may reflect a historically rapid

diversification process within this lineage or a limited number of informative sites in the COI sequences for this specific branch. However, the topology of the phylogenetic tree is corroborated by the haplotype network and species delimitation models (ABGD and ASAP), indicating that the low support value does not affect the validity of the inferred lineage pattern.

A total of 194 *R. sordida* COI sequences with an aligned length of 373 bp were obtained from 35 regions, primarily in China and Europe (Suppl. material 1: table S2). The number of polymorphic (segregating) sites (S) was 155, and the total number of mutations (Eta) was 267. These sequences comprised 117 haplotypes. Overall haplotype diversity was 0.9895, and total nucleotide diversity was 0.1121. Species delimitation analyses identified 43 genetic clades using the ABGD model, 58 clades using the ASAP model, and 70 clades using the bPTP model. The ASAP-derived classification showed closer congruence with both the phylogenetic topology and the actual geographic distribution of *R. sordida* and was therefore selected for downstream analyses. Among the ASAP-defined clades, Clade 23 contained the highest haplotype diversity (32 haplotypes), followed by Clade 7 (7 haplotypes) and Clades 6 and 22 (6 haplotypes each) (Table 1). Collectively, these results reveal high genetic diversity in both *A. beysunae* and *R. sordida*, with a substantial number of previously unrecognized clonal lineages that may be formally accepted as species following appropriate taxonomic procedures.

Phylogeography and population genetic differentiation of *A. beysunae*

Bayesian phylogenetic analyses showed that Clade 7, Clade 8, and Clade 10 were the largest lineages within Chinese *A. beysunae* populations (Fig. 1). These major clades were widely distributed across China and exhibited partially overlapping geographic distributions. Clade 8 was primarily distributed in eastern, central, and southern regions. Clade 10 encompassed populations from western, central, and southern regions, whereas Clade 7 displayed intermediate distributional characteristics, being concentrated in eastern, central, and southern areas. Notably, six minor clades were exclusively observed in island regions, including Hainan Island, Zhuhai's Wanshan Islands, and islands within Guangzhou's Pearl River estuary, suggesting potential local endemism (Fig. 2). Among these island clades, Clades 2, 3, 4, 5, and 9 each consisted of a single individual representing a unique haplotype. Clade 1 comprised three individuals, all sharing the same haplotype.

Haplotype network analysis revealed complex differentiation patterns in Chinese *A. beysunae* populations, characterized by three principal topologies: (1) star-like structures suggesting recent population expansion, with H16 potentially representing the ancestral haplotype; (2) tree-like configurations indicating gradual differentiation consistent with long-term population stability; and (3) reticulate networks implying potential convergent evolution. The remaining seven minor clades exhibited isolated branches and chain-like structures, reflecting deep evolutionary divergence (Suppl. material 1: fig. S1).

Neutrality tests yielded negative but statistically nonsignificant Tajima's *D* and Fu's *FS* values ($D = -0.55914$, $p = 0.305$; $FS = -8.09231$, $p = 0.116$), which do not support recent demographic expansion in Chinese *A. beysunae* populations (Table 1). Pairwise FST analysis identified significant genetic differentiation in 45 out of 120 population pairs, with the highest divergence observed between Kunming and Shanghai (FST = 0.737) and the lowest between Guangzhou and Zhuhai (FST = 0.093) (Suppl. material 1: table S4). AMOVA revealed limited genetic structure among the 15 populations (FST = 0.247, $p < 0.01$), with 24.7% of the total genetic variation attributed to differences among populations and 75.3% to variation within populations (Table 2). Mantel tests demonstrated a significant positive correlation between genetic divergence (FST) and geographic distance ($r = 0.477$, $p < 0.01$), suggesting that isolation by distance is an important driver of *A. beysunae* population differentiation in China.

Phylogeography and population genetic differentiation of *R. sordida*

Populations of *R. sordida* across Europe and Asia exhibited high phylogenetic diversity, with sympatric divergence suggested by overlapping distributions of distinct lineages (Fig. 3). Pronounced genetic differentiation separated European and Chinese lineages, with only Clade 6 shared between European and Chinese regions. European lineage composition varied substantially, with the UK harboring the highest diversity, including Clade 7, which was shared with Spain, and Clade 8, which was shared with Turkey, located at the interface between Europe and Asia. In China, Changde represented the hotspot of national diversity. The haplotype-rich Clade 23 was predominantly distributed across central, southern, and eastern China. Clade 22 spanned northeastern, eastern, central, and southwestern China, whereas the geographically widespread Clade 20 occurred throughout eastern, central, and northwestern China (Fig. 4).

Lineages associated with islands in the East and South China Seas exhibited distinct evolutionary trajectories. Clade 32 was restricted to Zhuhai and Sanya, corresponding to the Wanshan Archipelago and Hainan Island. Clade 28 occurred exclusively in Shantou and Haikou, representing Nanao Island and Hainan Island. Clade 29 was confined to Zhuhai's Wanshan Archipelago, and the Taiwan Island–endemic Clade 11 demonstrated strict isolation. These coastal island habitats—characterized by environmental homogeneity and geographic isolation from mainland populations—illustrate how island barriers drive genetic divergence (Fig. 4).

Table 1. Genetic diversity indices and neutrality test results for *A. beysunae* and *R. sordida* populations.

Species	Locality	Clade	Haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)	Tajima's *D*	p value	Fu's *Fs*	p value
A. beysunae	Beijing	C8	H63	\	\	0	1	0	\
A. beysunae	Lianyungang	C8, C7, C6	H20, H42, H41, H38, H40, H39	0.889	0.05487	-0.7452	0.231	3.68409	0.939
A. beysunae	Nanjing	C8, C10	H4, H3	0.667	0.04914	0	1	5.43808	0.972
A. beysunae	Shanghai	C8, C7	H23, H24, H25	0.7	0.03784	1.08859	0.848	4.84647	0.98
A. beysunae	Shantou	C8	H22	1	0.07617	0	1	3.43399	0.607
A. beysunae	Changde	C8, C7, C10	H61, H60, H59, H62, H56, H58, H57, H5	0.771	0.02502	-1.07508	0.112	4.13157	0.944
A. beysunae	Qingyuan	C8, C7, C10	H35, H33, H31, H37, H36, H30, H32, H29, H16, H34	0.884	0.04746	1.38411	0.951	4.39753	0.955
A. beysunae	Shaoguan	C8, C10	H20, H14, H16, H21, H17, H18, H19	0.781	0.02857	-0.02118	0.558	3.68722	0.928
A. beysunae	Guangzhou	C7, C10, C5	H54, H5, H34, H49, H44, H47, H50, H2, H48, H52, H1, H53, H46, H51	0.908	0.04847	-0.43529	0.371	5.25889	0.96
A. beysunae	Zhongshan	C10	H15	\	\	0	1	0	\
A. beysunae	Zhuhai	C10, C8, C2, C3	H12, H13, H14, H8, H11, H7, H9, H10	0.859	0.05768	0.32194	0.667	3.9447	0.962
A. beysunae	Haikou	C10, C9	H45, H43, H44	0.464	0.03054	-1.841	0.001	7.51157	0.994
A. beysunae	Sanya	C10, C1, C4	H26, H27, H28	0.7	0.05651	-0.45636	0.417	5.8799	0.99
A. beysunae	Dujiangyan	C10	H55	\	\	0	1	0	\
A. beysunae	Kunming	C10	H5, H6	0.4	0.00491	-1.12397	0.063	2.63906	0.884
A. beysunae	total			0.973	0.05341	-0.55914	0.305	-8.09231	0.116
R. sordida	Italy	C1, C4, C13	H1, H15, H19, H2	0.8	0.07846	1.2409	0.935	5.14921	0.976
R. sordida	Tanzania	C5	H3	\	\	0	1	0	\
R. sordida	Switzerland	C6	H4	\	\	0	1	0	\
R. sordida	Spain	C7	H5, H6	0.5	0.00134	-0.61237	0.377	0.17185	0.32
R. sordida	UK	C2, C3, C30, C7, C8, C9, C10, C18	H10, H11, H12, H22, H23, H24, H25, H26, H48, H49, H5, H50, H51, H52, H53, H54, H7, H8, H9	0.895	0.08887	1.21074	0.908	5.9238	0.977
R. sordida	Taiwan	C11	H13, H14	1	0.00536	0	1	0.69315	0.374
R. sordida	Australia	C14	H16	\	\	0	1	0	\
R. sordida	France	C15, C16	H17, H18	1	0.10188	0	1	3.63759	0.623
R. sordida	Turkey	C8, C17	H20, H21	0.6	0.04021	1.86069	0.996	8.2623	1
R. sordida	Shantou	C19, C27, C28, C35	H27, H44, H45, H59	1	0.11841	1.14036	0.85	1.95505	0.529
R. sordida	Nanjing	C20	H28	\	\	0	1	0	\
R. sordida	Dazhou	C21	H29	0	0	0	1	0	\
R. sordida	Guiyang	C22	H30, H31	1	0.00268	0	1	0	0.254
R. sordida	Guangzhou	C23, C50, C51, C52	H32, H34, H91, H92, H93	1	0.12681	0.7207	0.727	1.48044	0.473
R. sordida	Nanchang	C23	H33, H35, H40	0.833	0.01385	1.81983	0.944	1.5569	0.712
R. sordida	Huaian	C22	H36	0	0	0	1	0	\
R. sordida	Kunming	C12, C6, C24, C25	H37, H38, H78, H79, H80, H81	0.952	0.08822	0.19536	0.589	2.1881	0.783
R. sordida	Taian	C22	H39	0	0	0	1	0	\
R. sordida	Wuhan	C26	H41, H43	0.667	0.00179	0	1	0.20067	0.365
R. sordida	Zhoushan	C23	H42, H47	0.5	0.00134	-0.61237	0.365	0.17185	0.346
R. sordida	Zhuhai	C29, C31, C32	H46, H55, H56	0.833	0.12913	0.66805	0.775	5.45758	0.959
R. sordida	Zhanjiang	C33	H57	\	\	0	1	0	\
R. sordida	Wulumuqi	C34	H58	\	\	0	1	0	\
R. sordida	Shanghai	C23, C24, C25, C36	H60, H61, H62, H63	0.9	0.13941	1.06623	0.835	4.49568	0.94
R. sordida	Sanya	C37, C38, C32	H64, H65, H66	0.833	0.09383	0.72403	0.791	4.85556	0.957
R. sordida	Qingdao	C20	H67	\	\	0	1	0	\
R. sordida	Lianyungang	C39, C40, C41, C42, C43, C44, C45, C24, C34	H58, H68, H69, H70, H71, H72, H73, H74, H75, H76, H77	0.956	0.11449	1.04813	0.876	2.38164	0.881
R. sordida	Kuche	C20	H82, H83	1	0.01072	0	1	1.38629	0.458
R. sordida	Kashi	C46	H84	0	0	0	1	0	\
R. sordida	Haikou	C6, C28, C47, C48	H85, H86, H87, H88, H89	0.905	0.09613	0.99725	0.881	4.62054	0.962
R. sordida	Haerbin	C49	H90	\	\	0	1	0	\
R. sordida	Dujiangyan	C6	H94	\	\	0	1	0	\
R. sordida	Changsha	C23, C25, C53, C54, C55, C20	H100, H67, H95, H96, H97, H98, H99	1	0.1352	0.90396	0.842	0.71084	0.432

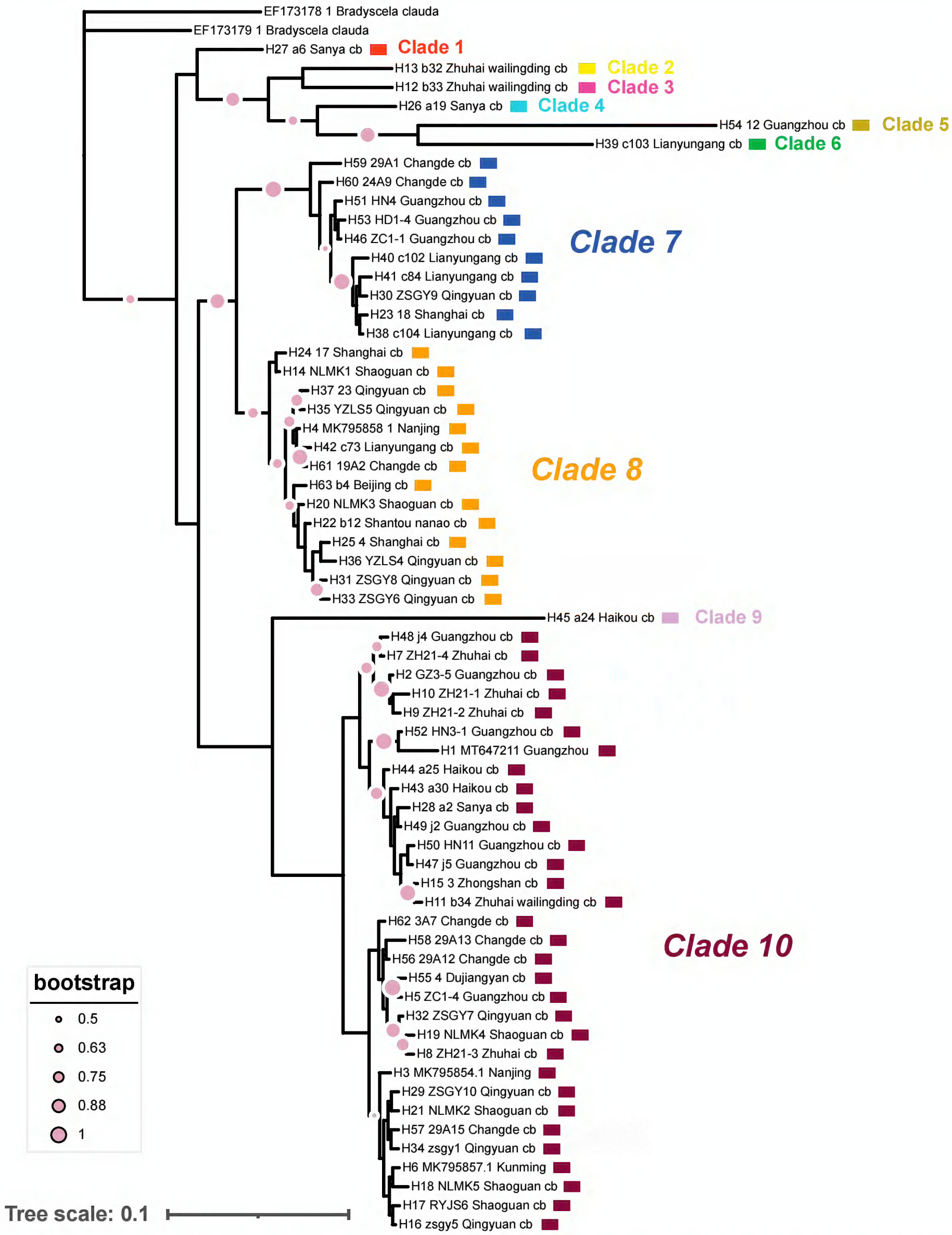

Figure 1. Bayesian phylogenetic tree of the *Adineta beysunae* species complex in China based on mitochondrial COI sequences. A single representative sequence of each haplotype was included in the tree. Boxes with different colors represent clades identified by the ABGD and ASAP models.

Haplotype network analysis revealed complex phylogenetic structuring in worldwide *R. sordida* populations (Suppl. material 1: fig. S2). The tree-like topology, characterized by limited central haplotypes and elongated branching patterns, reflects gradual differentiation driven by accumulated mutations. Numerous intermediate haplotypes inferred from the network indicate substantial undiscovered diversity, likely attributable to the species' broad geographic distribution and incomplete sampling coverage. Regional substructures further revealed

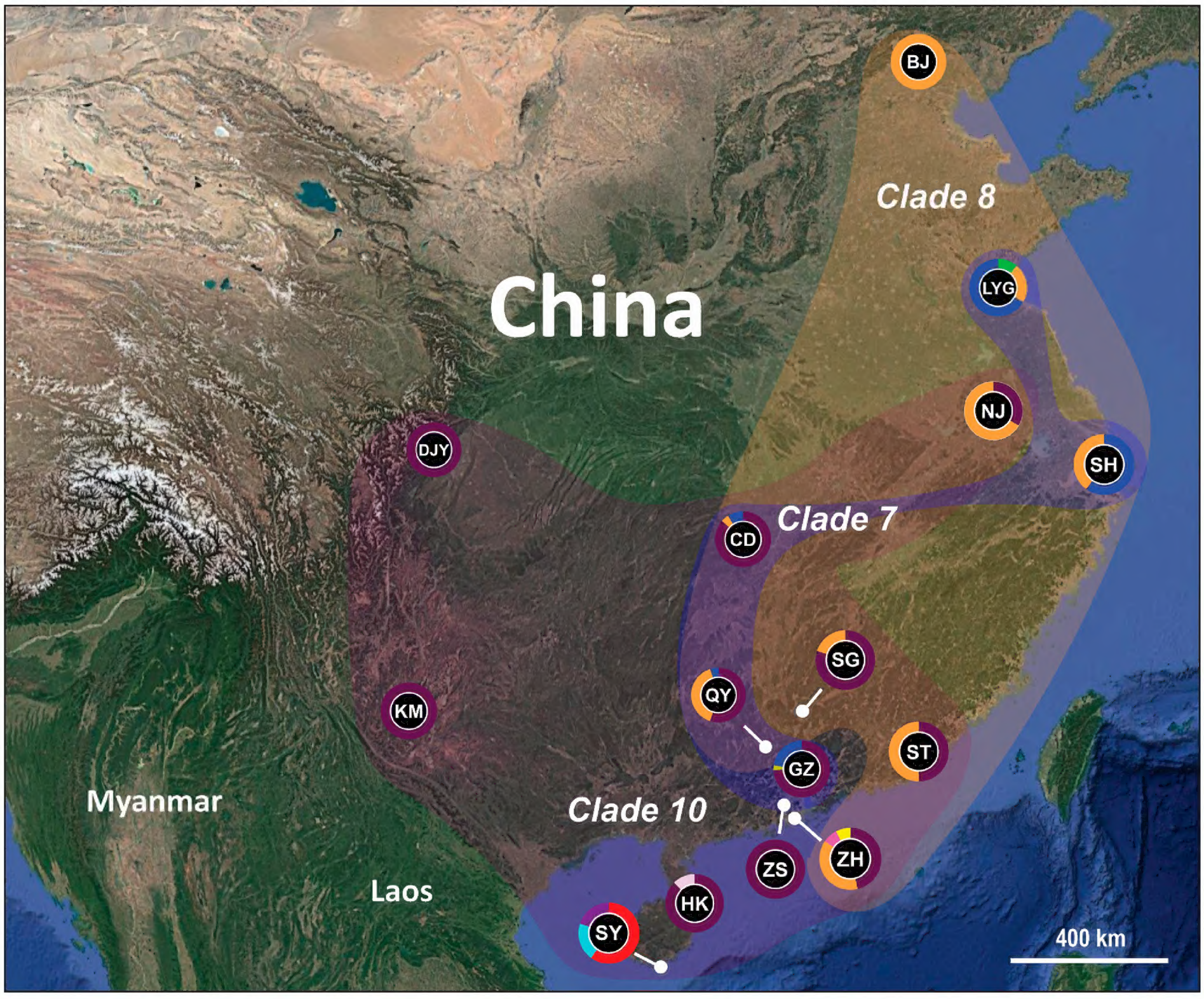

Figure 2. Distribution of the *Adineta beysunae* species complex in China. Rings of different colors represent the number of sequences belonging to different clades. The primary clades (Clades 7, 8, and 10) are highlighted on the map, with their distribution ranges indicated by shaded areas.

Table 2. Analysis of molecular variance (AMOVA) within and among populations of *Adineta beysunae*.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation indices	*p* value
Among populations	14	467.943	2.77366	24.74	0.24735	0.00000
Within populations	130	1097.16	8.43969	75.26		

heterogeneous evolutionary dynamics. Linear chain-like configurations, such as H112–H68–H93 across central, eastern, and southern China, suggest progressive mutation accumulation potentially linked to slow population expansion or directional selection. In contrast, closed-loop clusters, including terminal haplotypes in southern China and the H38–H95–H98 complex in central and southwestern China, likely arose from recurrent mutations. Together, these patterns underscore spatially divergent evolutionary trajectories shaping the global genetic architecture of *R. sordida*.

Neutrality tests (Tajima's D = 1.81542, p = 0.905; Fu's FS = −23.75605, p = 0.002) suggest that Chinese *R. sordida* populations may have undergone a recent demographic expansion (Table 1). AMOVA revealed significant genetic differentiation among the 35 worldwide populations (FST = 0.244, $p < 0.01$), with 24.5% of total genetic variation attributed to differences among populations and 75.5% to within-population variation, indicating limited geographic structuring (Table 3). Pairwise FST analysis identified significant differentiation in 166 population pairs, ranging from 0.992 (Spain vs. Taian) to 0.095 (Lianyungang vs. Changde) (Suppl. material 1: table S5). Mantel tests showed no significant correlation between genetic divergence (FST) and geographic distance (r = 0.148, p = 0.122), indicating that geographic distance exerted a relatively limited influence on the distribution of *R. sordida* lineages across China, potentially reflecting natural or anthropogenic dispersal processes or broad environmental tolerance.

Table 3. Analysis of molecular variance (AMOVA) within and among populations of *Rotaria sordida*.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation indices	*p* value
Among populations	34	1458.52	5.24345	24.45	0.24447	0
Within populations	159	2576.598	16.20502	75.55		

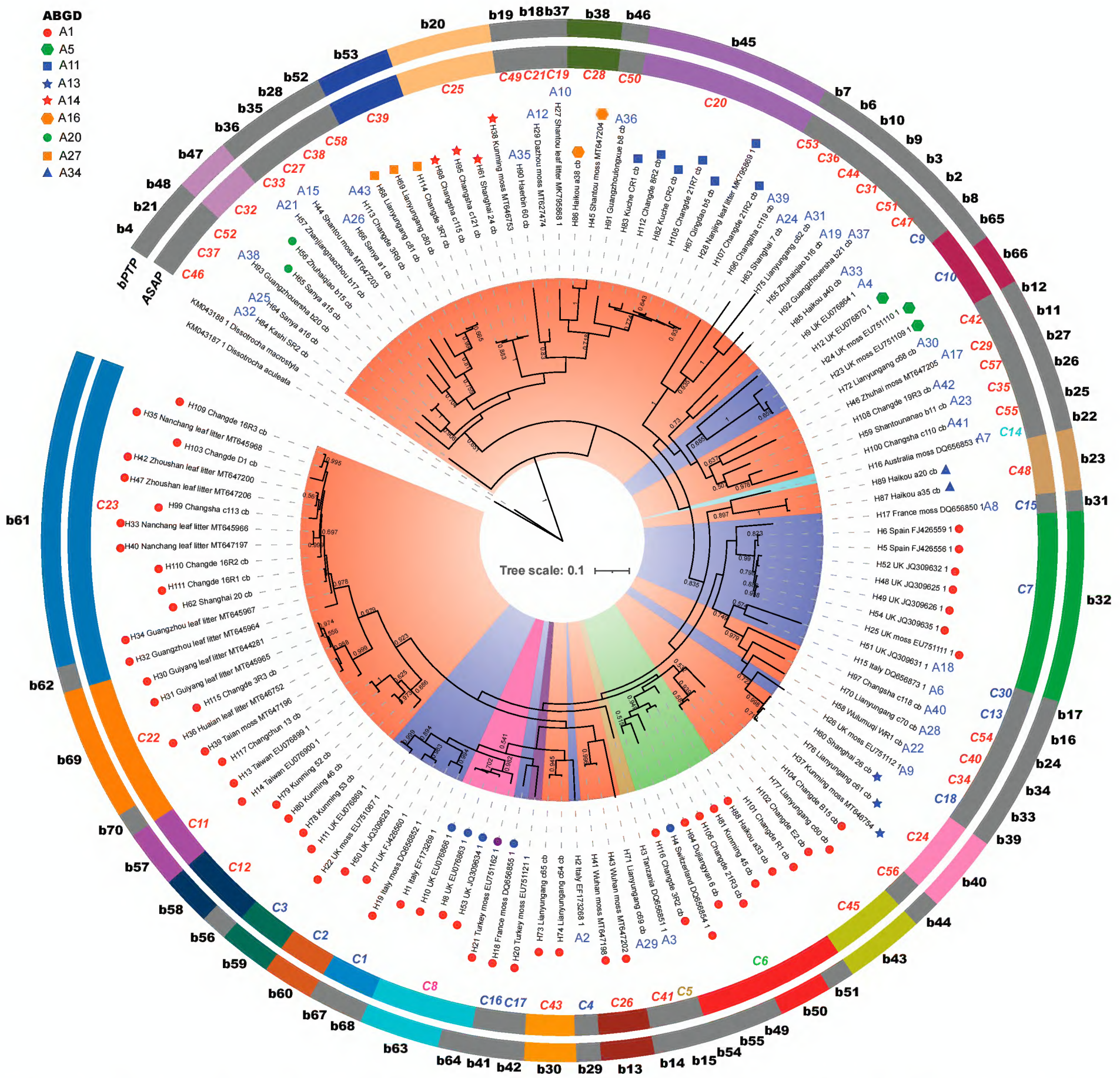

Figure 3. Bayesian phylogenetic tree of the *Rotaria sordida* species complex in China and worldwide based on mitochondrial COI sequences. A single representative sequence of each haplotype was included in the tree. Outer rings with different colors represent clades identified by the ASAP and bPTP models. Nodes positioned behind the sequence names represent clades detected by the ABGD model. The colors of the inner ring indicate the geographic origin of sequences: red, China; blue, Europe; green, Clade 6 present in both China (red nodes in front of the sequence name) and Europe (blue nodes in front of the sequence name); pink, Clade 8 present in both Turkey (purple nodes in front of the sequence name) and Europe (blue nodes in front of the sequence name); purple, Turkey; light blue, Australia; and brown, Tanzania

Driving factors of phylogeographic patterns

Longitude, latitude, elevation, precipitation, temperature, wind speed, GDP, population density, and sampling intensity (number of analyzed sequences) were assessed as potential drivers of phylogeographic patterns in *Adineta beysunae* and *Rotaria sordida*, categorized into spatial, climatic, and anthropogenic factors. Linear regression analyses revealed a significant positive correlation between pairwise FST values and geographic

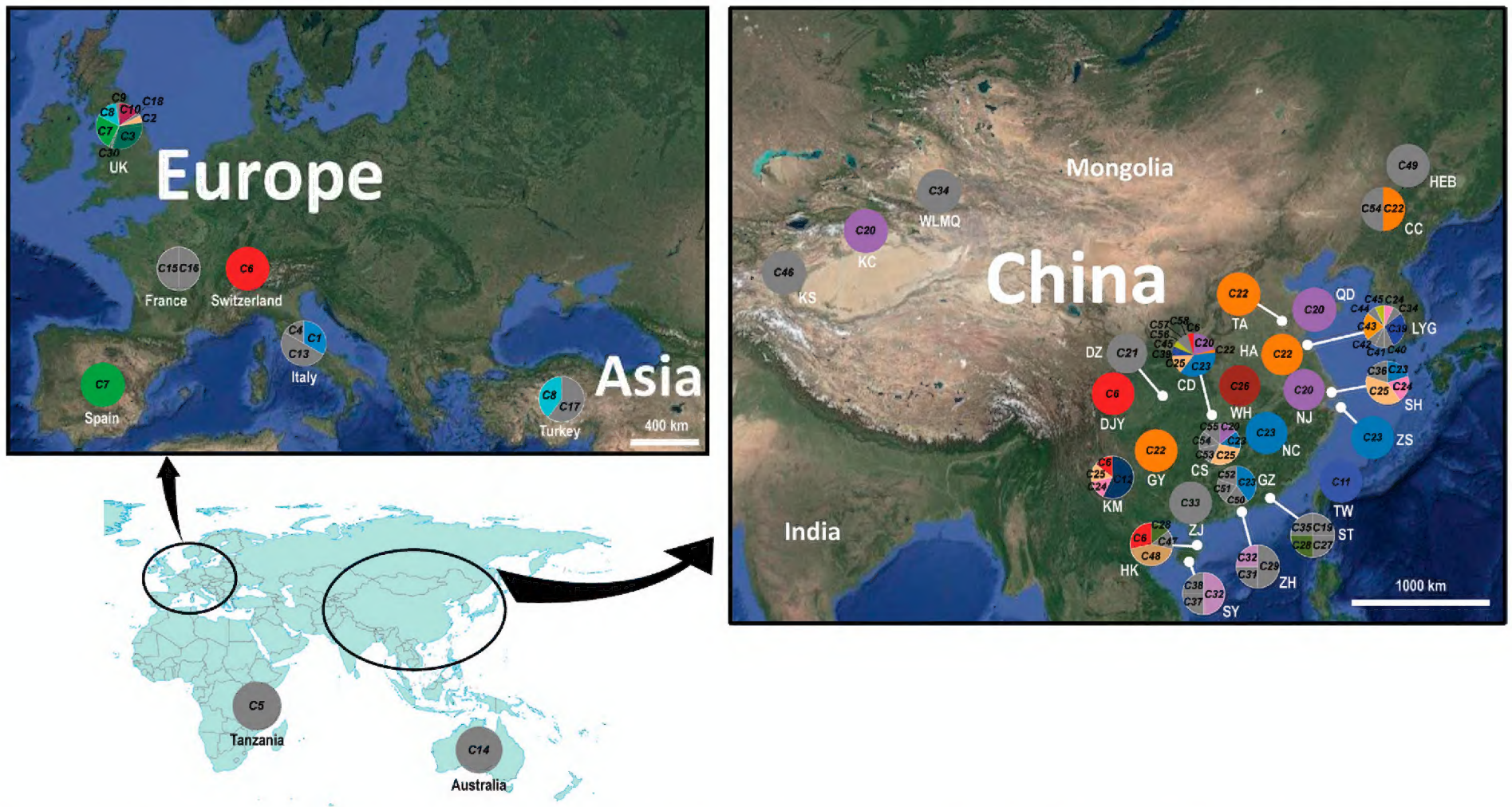

Figure 4. Distribution of the *Rotaria sordida* species complex in China and worldwide. Sectors of different colors represent the number of sequences belonging to different clades. Grey indicates clades that occur at only one locality.

distance for both species ($p < 0.01$), although the explanatory power (R^2) remained relatively low. Spatial factors exerted a stronger influence on lineage divergence in *A. beysunae* than in *R. sordida* (Fig. 5A, B). Hierarchical partitioning analysis based on dbRDA identified climatic variables (53.7%) as the dominant drivers of phylogeographic structure in the *A. beysunae* species complex, followed by spatial (36.05%) and anthropogenic factors (10.3%) (Suppl. material 1: table S6). Temperature seasonality accounted for the highest proportion of explained variance (13.16%), followed by mean wind speed (11.68%), longitude (11.23%), and elevation (11.04%). Among anthropogenic variables, GDP contributed the most (8.27%), whereas sampling intensity showed the lowest contribution (4.29%) (Fig. 5C; Suppl. material 1: table S6). For Chinese populations of *R. sordida*, climatic factors explained 59.8% of the total variance in phylogeographic structure, followed by spatial factors (35.07%) and anthropogenic influences (5.11%) (Suppl. material 1: table S6). Among spatial variables, longitude exhibited the strongest contribution (12.48%), followed by elevation (11.31%). Climatic drivers were dominated by precipitation seasonality (11.26%) and annual mean temperature (11.22%). Anthropogenic effects showed comparable contributions from population density (6.58%) and GDP (6.13%), whereas sampling intensity had minimal explanatory power (1.40%) (Fig. 5D; Suppl. material 1: table S6). Collectively, these results quantitatively demonstrate the predominant role of climatic heterogeneity in shaping the genetic patterns of *A. beysunae* and *R. sordida*, with secondary but non-negligible effects of geographic gradients.

Discussion

High genetic diversity in bdelloid rotifers *A. beysunae* and *R. sordida*

This study presents the first comprehensive phylogeographic analysis of the *A. beysunae* and *R. sordida* species complexes across a broad geographic range in China and worldwide. Based on COI phylogenetic reconstruction combined with subsequent delimitation using the ASAP model, *A. beysunae* and *R. sordida* were divided into 10 and 58 genetic clades, respectively. The clades of *R. sordida* comprised 41 from China, 12 from Europe, one shared between China and Europe, one shared between Turkey and Europe, one from Turkey, one from Tanzania, and one from Australia. These findings substantially expand the known genetic diversity of *R. sordida* relative to earlier studies. For example, Fontaneto et al. (2008, 2009) reported 33 haplotypes and 17 cryptic species based on 50 COI sequences, whereas our results reveal 41 additional lineages, exceeding even the most clade-rich bdelloid species complexes previously reported (e.g., *A. vaga*: 36 clades; Fontaneto et al. 2011).

Rotaria sordida, notably, exhibited markedly higher genetic diversity than *A. beysunae*, with greater cryptic species richness and haplotype variation. This pattern is consistent with habitat preference analyses reported by Wang et al. (2023b), which demonstrated divergent ecological strategies between the two species complexes. In the present study, most *A. beysunae* individuals were collected from leaf litter, with only a small proportion found in moss. In contrast, *R. sordida* occurred in roughly equal numbers in moss and litter and was also detected in

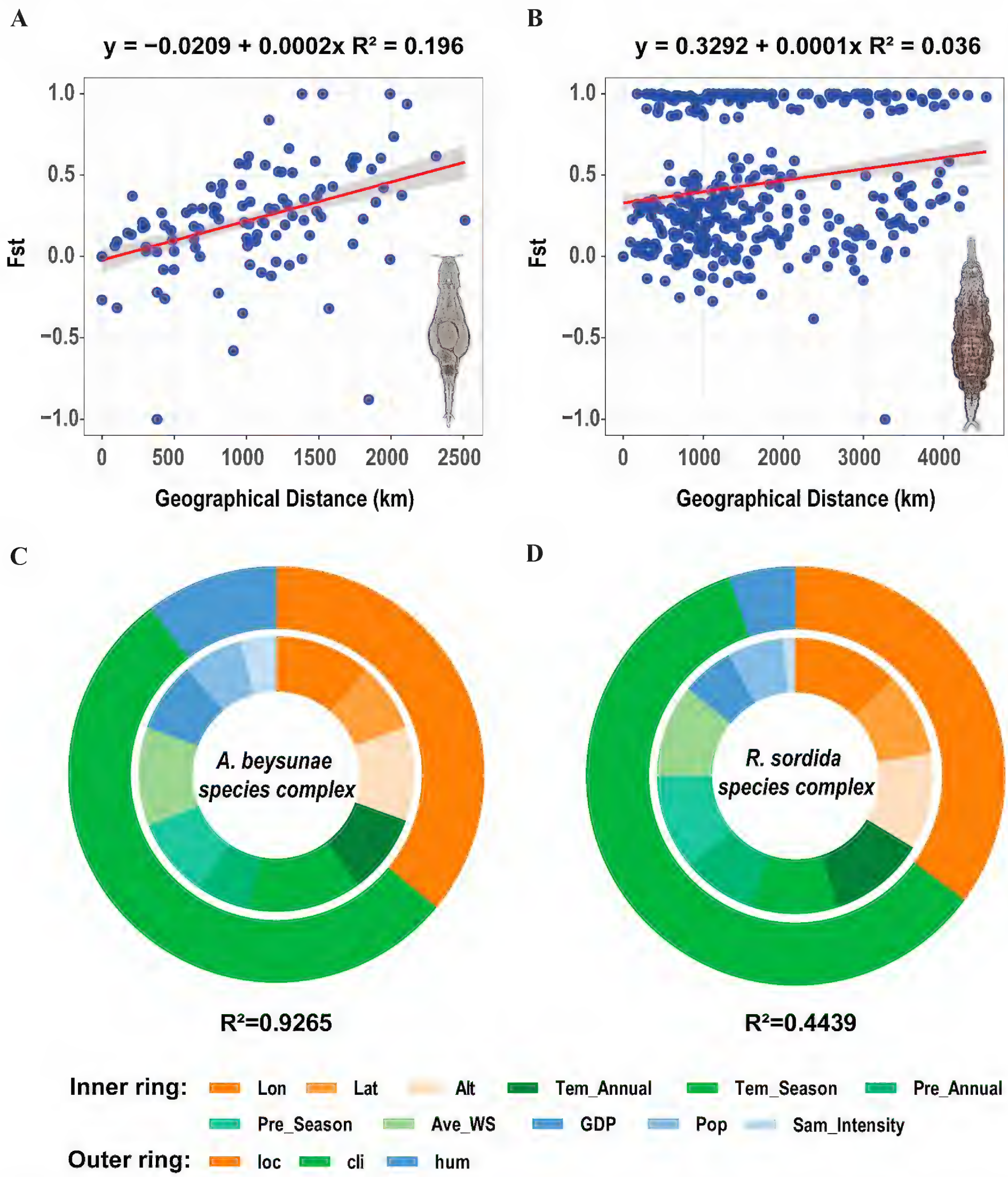

Figure 5. Linear regression analysis of FST values for *A. beysunae* (**A**) and *R. sordida* (**B**) populations in relation to geographic distance. Hierarchical partitioning analysis of driving factors for *A. beysunae* (**C**) and *R. sordida* (**D**) populations. The area of each ring represents the percentage of variance explained by each factor. Abbreviations: Lon, longitude; Lat, latitude; Alt, altitude; Tem_Annual, annual mean temperature; Tem_Season, temperature seasonality; Pre_Annual, annual precipitation; Pre_Season, precipitation seasonality; Ave_WS, average wind speed; GDP, Gross Domestic Product; Pop, population density; Sam_Intensity, sampling intensity; loc, spatial factors; cli, climatic factors; hum, anthropogenic factors.

soil. These habitats provide distinct nutritional resources and ecological niches, which may promote lineage persistence and diversification (Walczyńska et al. 2024). However, the present study could not further quantify the role of microhabitat variation in driving genetic differentiation, primarily due to the absence of measured environmental parameters (e.g., nutrient composition, pH, and moisture content). Moreover, even within the same broad habitat category (e.g., "moss" or "litter"), the specific conditions at different sites—such as sun exposure,

humidity, substrate type (soil, rock, or bark for moss), or litter composition (coniferous vs. broadleaf)—can create vastly different microenvironments. Future studies should therefore move beyond qualitative habitat descriptions and incorporate quantitative microenvironmental measurements to better assess how microhabitat heterogeneity influences bdelloid rotifer genetic diversity.

A combined widespread and localized phylogeographic pattern in *A. beysunae* and *R. sordida*

Our results support the hypothesis that *A. beysunae* and *R. sordida* exhibit a combined widespread and localized phylogeographic pattern. Their cryptic species include both geographically restricted lineages, such as those confined to islands, and broadly distributed lineages, including transcontinental lineages (e.g., Clade 6 of the *R. sordida* species complex spanning Europe and China). Regionally restricted lineages, particularly in island ecosystems, are likely shaped by geographic isolation and environmental heterogeneity (Santamaria 2019). Oceanic barriers restrict mainland–island gene flow, while niche differentiation driven by heterogeneous resource availability accelerates divergence, a process that may be amplified in asexual taxa such as bdelloid rotifers (Fontaneto et al. 2009; Liang et al. 2022; Walczyńska et al. 2024). Notably, island-associated *R. sordida* lineages, including Clade 28 (ST–HK) and Clade 32 (ZH–SY), were not detected on adjacent mainland sites but showed evidence of dispersal between islands, suggesting a role for mainland–island environmental differences in shaping lineage distributions (Woolfit and Bromham 2005). Outside island systems, major lineages exhibited limited distributional divergence, with overlapping ranges despite regional tendencies (Zhu et al. 2025). Previous studies reported pronounced micro-endemism in extreme environments such as the Qinghai–Tibet (e.g., mitochondrial clades of *Brachionus plicatilis* and both mitochondrial and nuclear clades of *R. rotatoria* (Deng et al. 2022a; Zhu et al. 2025), a pattern also observed in plateau-adapted crustaceans exhibiting morphological divergence (e.g., larger body size; Ma et al. 2020). Although similar adaptations were anticipated in Qinghai-Tibet Plateau rotifers, low population densities limited genetic sampling of high-altitude lineages in the present study. In contrast, the absence of strong geographic barriers in eastern, southern, and central China likely facilitates lineage dispersal, resulting in overlapping distributions without pronounced spatial structuring, consistent with patterns reported for eastern Chinese populations of *B. calyciflorus* and *R. rotatoria* (Xiang et al. 2011; Yang et al. 2022; Zhu et al. 2025).

Globally, the morphospecies *R. sordida* is distributed across Eurasia, with additional records from Africa and Oceania (Segers 2007; Fontaneto et al. 2008; Cakil et al. 2021). However, lineage composition within the *R. sordida* species complex differs markedly between Europe and China, with only Clade 6 shared between the two regions. This lineage spans a minimum straight-line distance of approximately 7,600 km from Central Europe (Switzerland) to China (DJY), making it the most widely distributed lineage detected in this study. Within China, it occurs mainly in southwestern and southern regions. Several other clades, including Clade 20, Clade 22, Clade 23, and Clade 25, also show broad national distributions, with maximum recorded distances reaching approximately 3,300 km (between KC and QD) for Clade 20 and 2,545 km (between GY and CC) for Clade 22. These widespread lineages span multiple climatic zones and distinct geographic units, indicating a history of long-distance dispersal combined with broad ecological tolerance (Walczyńska et al. 2024). Similarly, within China, clades of the *A. beysunae* complex—Clade 7, Clade 8, and Clade 10—also exhibit wide distributions. However, the spatial ranges of these widespread clades are not fully congruent and show partially overlapping but distinct distributional tendencies, a pattern more pronounced in *A. beysunae*. This indicates that even widespread lineages are not truly ubiquitous but may instead be cosmopolitan (e.g., transcontinental) or broadly distributed within a particular region (e.g., across multiple provinces in China) (Fontaneto et al. 2008). Furthermore, within China, 29 clades of *R. sordida* and 7 clades of *A. beysunae* were each restricted to a single sampling locality, representing extreme cases of localized lineages. Of these, 21 and 6 clades, respectively, were represented by only one individual. Consequently, future studies should expand both geographic sampling and individual coverage to more comprehensively and systematically resolve the geographic distribution patterns of bdelloid rotifers in China and worldwide.

Environment-dominated multi-driver framework shapes phylogeography of bdelloid rotifers

Understanding the drivers of biodiversity is pivotal for revealing the mechanisms underlying diversity patterns. Although substantial progress has been made in elucidating species-level diversity, the forces shaping phylogeographic distributions remain poorly resolved. Our findings demonstrate that climatic factors, rather than geographic isolation, explain most of the genetic variation in Chinese *A. beysunae* and *R. sordida* populations. This result highlights the dominant role of environmental factors in shaping genetic diversity patterns in rotifers (Zhu et al. 2025). Within morphospecies complexes, distinct lineages often exhibit restricted and specific niche requirements (Fontaneto et al. 2009; Wang et al. 2023b).

Fontaneto et al. (2007) investigated trophic niche divergence in eight *Rotaria* species inhabiting distinct microhabitats, including rivers, ponds, moss, and lichen, by correlating trophi morphology with genetic differentiation. That study revealed pronounced trophi divergence among morphospecies—likely reflecting adaptation to

ecological niches—despite limited divergence among genetic clades, suggesting niche-driven speciation in asexual bdelloids. Additionally, sympatric divergence was detected in *R. macrura* populations. Comparable patterns have been reported in other rotifers, in which co-occurring lineages exhibit divergent life-history strategies under environmental stress. For example, Xiang et al. (2016a) showed that three sympatrically differentiated *R. rotatoria* lineages displayed distinct life-history responses to elevated temperatures. The interactive effects of cryptic species identity and temperature significantly influenced life-history parameters of *R. rotatoria*, including juvenile period, reproductive period, and mean lifespan. Similar patterns have been documented in monogonont rotifers: two cryptic *Brachionus calyciflorus* species in tropical shallow lakes coexist via divergent strategies—high survival with slow population growth vs. rapid population growth with low survival—under relatively high temperatures and oligotrophic conditions (Wang et al. 2014). Notably, different cryptic species, or geographic strains within the same cryptic species originating from contrasting climates, exhibit differential tolerance to climatic factors such as temperature (Xiang et al. 2016b, 2024; Li et al. 2024), highlighting environmental selection as a key filter for lineage persistence. Collectively, these studies provide multi-scale biogeographic evidence linking niche divergence to cryptic speciation. In addition, contemporary phylogeographic patterns of bdelloid rotifers reflect historical processes of dispersal and adaptation associated with climatic oscillations. For instance, Shain et al. (2024) identified widespread cryophilic bdelloid populations in Norwegian glacial habitats, where mitochondrial and nuclear markers delineated 16 lineages tracing back to a colonization event approximately 2.58 million years ago. These rotifers exhibit exceptional freeze–thaw tolerance, enabling persistence under extreme conditions. Investigations in New Zealand thermal springs and glaciers further revealed that extreme micro-metazoan communities derive from both local adaptation of mesophilic species and dispersal from distant thermal or cryogenic habitats (Shain et al. 2022). When integrated with our results, these findings indicate that climatic factors (53.7% for *A. beysunae*; 59.8% for *R. sordida*) and spatial variables (36.05% for *A. beysunae*; 35.07% for *R. sordida*) jointly shape the phylogeographic patterns of Chinese *A. beysunae* and *R. sordida* populations, with climate exerting a predominant selective force. A similar multi-factor-driven mechanism has been reported for Chinese populations of *R. rotatoria* (Zhu et al. 2025).

Among climatic variables, temperature appears to exert the strongest influence on rotifer genetic variation, as supported by controlled thermal stress experiments. Elevated temperatures reduce the fitness of thermally sensitive lineages and are accompanied by distinct gene expression profiles (Li et al. 2024). Tropical lineages employ adaptive strategies under heat stress, such as downregulation of metabolic genes and reallocation of resources toward reproduction, thereby maintaining population persistence under thermal extremes (Li et al. 2024). In contrast, lineages adapted to cold plateau environments exhibit enhanced reproductive capacity under low-temperature conditions, demonstrating ecological responses distinct from those of lineages originating from temperate zones (Zhu et al. 2025). Wind speed and precipitation also play critical roles. Wind facilitates long-distance dispersal of bdelloid rotifers and may aid escape from fungal pathogens (Wilson and Sherman 2010, 2013; Rivas Jr et al. 2019). During diapausing stages, these desiccation-resistant animals—behaving as particles similar to dust grains—can be transported by storms (Rivas et al. 2019), while precipitation mediates their deposition into terrestrial habitats (e.g., moss, leaf litter, and soil) (Moreno et al. 2016). Moreover, water availability is essential for bdelloid rotifer survival. Dehydration induces body contraction and entry into a dormant state until rehydration occurs (Ricci 2017). Consequently, precipitation levels directly influence population abundance and community composition.

Beyond macroclimatic conditions, microhabitat factors, including nutrient availability and moisture content, also strongly influence rotifer lineage distributions. In lakes hosting multiple cryptic species, seasonal succession among lineages reflects niche partitioning along temporal and trophic axes driven by differential resource requirements. For example, Ortells et al. (2003) documented pronounced seasonal turnover within the *Brachionus plicatilis* cryptic species complex, whereas cryptic species of monogonont rotifers such as *Synchaeta* and *Keratella* exhibit species-specific nutrient requirements (Obertegger et al. 2012; Gabaldón et al. 2017). The present study did not quantify microhabitat-level environmental variables, which precluded analyses of trophic niche differentiation at fine spatial scales. Future investigations should therefore employ targeted sampling strategies to disentangle the relative contributions of climatic drivers (e.g., temperature seasonality) versus localized factors (e.g., nutrient concentrations, moisture gradients, microbial community composition) in shaping lineage distributions.

Among spatial factors, longitude and elevation play important roles in population genetic differentiation. This pattern likely reflects pronounced longitudinal gradients in geomorphology combined with substantial altitudinal variation across China, which together create dispersal barriers between geographic regions (Zhu et al. 2025). Studies of monogonont rotifers and crustacean lineages across China consistently show that eastern plains harbor diverse lineages, whereas western plateaus and mountainous regions support distinct genetic clusters (Deng et al. 2022a, 2022b; Yang et al. 2022; Wang et al. 2023a; Zhu et al. 2025).

Human activities also influence contemporary species dispersal and distribution, with microscopic organisms often transported inadvertently through human movement. At the same time, increasing anthropogenic pressure imposes selective filters on species survival and establishment. In this study, population density and GDP were used as proxies for the intensity of human activity.

The results indicate that anthropogenic factors had the weakest explanatory power among the tested drivers, consistent with findings by Partemi et al. (2024), who detected no significant negative effects of urbanization on species or genetic diversity.

In addition to the drivers examined here, geological and historical climatic events—including uplift of the Tibetan Plateau (Zhu et al. 2025), continental drift and vicariance (Cakil et al. 2021), and cycles of glacial and interglacial periods (Shain et al. 2024)—as well as biological vectors such as migratory birds and mammals (Moreno et al. 2019; Zhu et al. 2025), have profoundly influenced present-day phylogeographic patterns of micro-invertebrates. Phylogeographic pattern formation therefore represents a complex process driven by multiple interacting factors. To fully elucidate the mechanisms shaping micro-invertebrate biogeography, future research should integrate these drivers into quantifiable mechanistic models and test them across diverse invertebrate taxa, including bdelloid rotifers.

Conclusion

Using mitochondrial COI marker sequences, this study conducted a large-scale analysis of genetic diversity and phylogeographic patterns in two bdelloid rotifer species complexes, *A. beysunae* and *R. sordida*, across China. We identified multiple widespread lineages with overlapping distributions, alongside numerous endemic lineages restricted to specific localities, including islands. Phylogeographic structuring was predominantly shaped by climatic factors, notably seasonal temperature variation, annual precipitation, and mean wind speed, which exerted stronger influences than geographic parameters, such as longitude and elevation, and human activities. Collectively, these findings highlight the substantial cryptic diversity of Chinese *A. beysunae* and *R. sordida* populations, expanding our understanding of their regional and global distributions and species richness. This work provides foundational data from China for future global-scale evolutionary studies on bdelloid rotifer population dynamics.

Data archiving

All newly generated sequences have been deposited in GenBank under accession numbers PX451973–PX452078 (*A. beysunae*) and PX452102–PX452159 (*R. sordida*).

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References

Burgazzi G, Laini A, Ovaskainen O, Saccò M, Stubbington R, Viaroli P (2020) Communities in high definition: Spatial and environmental factors shape the micro-distribution of aquatic invertebrates. Freshwater Biology 65(12): 2053–2065. https://doi.org/10.1111/fwb.13599

Cakil ZV, Garlasché G, Iakovenko N, Di Cesare A, Eckert EM, Guidetti R, Hamdan L, Janko K, Lukashanets D, Rebecchi L, Schiaparelli S, Sforzi T, Kašparová EŠ, Velasco-Castrillón A, Walsh EJ, Fontaneto D (2021) Comparative phylogeography reveals consistently shallow genetic diversity in a mitochondrial marker in Antarctic bdelloid rotifers. Journal of Biogeography 48(7): 1797–1809. https://doi.org/10.1111/jbi.14116

Clement M, Posada DCKA, Crandall KA (2000) TCS: A computer program to estimate gene genealogies. Molecular Ecology 9(10): 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

De Luca D, Piredda R, Sarno D, Kooistra WH (2021) Resolving cryptic species complexes in marine protists: Phylogenetic haplotype networks meet global DNA metabarcoding datasets. The ISME Journal 15(7): 1931–1942. https://doi.org/10.1038/s41396-021-00895-0

Deng Z, Yang W, Blair D, Hu W, Yin M (2022a) Diversity of *Brachionus plicatilis* species complex (Rotifera) in inland saline waters from China: Presence of a new mitochondrial clade on the Tibetan Plateau. Molecular Phylogenetics and Evolution 171: 107457. https://doi.org/10.1016/j.ympev.2022.107457

Deng Z, Yao Y, Blair D, Hu W, Yin M (2022b) *Ceriodaphnia* (Cladocera: Daphniidae) in China: Lineage diversity, phylogeography and possible interspecific hybridization. Molecular Phylogenetics and Evolution 175: 107586. https://doi.org/10.1016/j.ympev.2022.107586

Donner J (1965) Ordnung Bdelloidea (Rotatoria, Rädertiere). Akademie-Verlag, Berlin, 267 pp.

Dunthorn M, Stoeck T, Wolf K, Breiner HW, Foissner W (2012) Diversity and endemism of ciliates inhabiting Neotropical phytotelmata. Systematics and Biodiversity 10(2): 195–205. https://doi.org/10.1080/14772000.2012.685195

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10(3): 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fick SE, Hijmans RJ (2017) WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37(12): 4302–4315. https://doi.org/10.1002/joc.5086

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Fontaneto D (2011) Biogeography of Microscopic Organisms: Is Everything Small Everywhere? Cambridge University Press, Cambridge, 365 pp. https://doi.org/10.1017/CBO9780511974878

Fontaneto D, Herniou EA, Boschetti C, Caprioli M, Melone G, Ricci C, Barraclough TG (2007) Independently evolving species in asexual bdelloid rotifers. PLOS Biology 5(4): e87. https://doi.org/10.1371/journal.pbio.0050087

Fontaneto D, Barraclough TG, Chen K, Ricci C, Herniou EA (2008) Molecular evidence for broad-scale distributions in bdelloid rotifers: Everything is not everywhere but most things are very widespread. Molecular Ecology 17(13): 3136–3146. https://doi.org/10.1111/j.1365-294X.2008.03806.x

Fontaneto D, Kaya M, Herniou EA, Barraclough TG (2009) Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. Molecular Phylogenetics and Evolution 53(1): 182–189. https://doi.org/10.1016/j.ympev.2009.04.011

Fontaneto D, Iakovenko N, Eyres I, Kaya M, Wyman M, Barraclough TG (2011) Cryptic diversity in the genus *Adineta* Hudson & Gosse, 1886 (Rotifera: Bdelloidea: Adinetidae): a DNA taxonomy approach. Hydrobiologia 662(1): 27–33. https://doi.org/10.1007/s10750-010-0481-7

Fontaneto D, Eckert EM, Aninic N, Lara E, Mitchell EA (2019) We are ready for faunistic surveys of bdelloid rotifers through DNA barcoding: The example of Sphagnum bogs of the Swiss Jura Mountains. Limnetica 38(1): 213–225. https://doi.org/10.23818/limn.38.02

Freckman DW, Virginia RA (1993) Extraction of nematodes from Dry Valley Antarctic soils. Polar Biology 13(7): 483–487. https://doi.org/10.1007/BF00233139

Gabaldón C, Fontaneto D, Carmona MJ, Montero-Pau J, Serra M (2017) Ecological differentiation in cryptic rotifer species: What we can learn from the *Brachionus plicatilis* complex. Hydrobiologia 796(1): 7–18. https://doi.org/10.1007/s10750-016-2723-9

Guidetti R, Massa E, Bertolani R, Rebecchi L, Cesari M (2019) Increasing knowledge of Antarctic biodiversity: New endemic taxa of tardigrades (Eutardigrada; Ramazzottiidae) and their evolutionary relationships. Systematics and Biodiversity 17(6): 573–593. https://doi.org/10.1080/14772000.2019.1649737

Holman LE, De Bruyn M, Creer S, Carvalho G, Robidart J, Rius M (2021) Animals, protists and bacteria share marine biogeographic patterns. Nature Ecology & Evolution 5(6): 738–746. https://doi.org/10.1038/s41559-021-01439-7

Iakovenko NS, Smykla J, Convey P, Kašparová E, Kozeretska IA, Trokhymets V, Dykyy I, Plewka M, Devetter M, Duriš Z, Janko K (2015) Antarctic bdelloid rotifers: Diversity, endemism and evolution. Hydrobiologia 761(1): 5–43. https://doi.org/10.1007/s10750-015-2463-2

Rivas Jr JA, Schröder T, Gill TE, Wallace RL, Walsh EJ (2019) Anemochory of diapausing stages of microinvertebrates in North American drylands. Freshwater Biology 64(7): 1303–1314. https://doi.org/10.1111/fwb.13306

Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Lai J, Zou Y, Zhang J, Peres-Neto PR (2022) Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. Methods in Ecology and Evolution 13(4): 782–788. https://doi.org/10.1111/2041-210X.13800

Leigh JW, Bryant D (2015) popart: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9): 1110–1116. https://doi.org/10.1111/2041-210X.12410

Leigh DM, Hendry AP, Vázquez-Domínguez E, Friesen VL (2019) Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. Evolutionary Applications 12(8): 1505–1512. https://doi.org/10.1111/eva.12810

Letunic I, Bork P (2021) Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. Nucleic Acids Research 49(W1): W293–W296. https://doi.org/10.1093/nar/gkab301

Li M, Gao F, Zhu L, Li J, Xiang J, Xi Y, Xiang X (2024) Geographic origin shapes the adaptive divergences of *Rotaria rotatoria* (Rotifera, Bdelloidea) to thermal stress: Insights from ecology and transcriptomics. Ecology and Evolution 14(4): e11307. https://doi.org/10.1002/ece3.11307

Liang D, McManus GB, Wang Q, Sun X, Liu Z, Lin S, Yang Y (2022) Genetic differentiation and phylogeography of rotifer *Polyarthra dolichoptera* and *P. vulgaris* populations between Southeastern China and eastern North America: High intercontinental differences. Ecology and Evolution 12(5): e8912. https://doi.org/10.1002/ece3.8912

Ma X, Ni Y, Wang X, Hu W, Yin M (2020) Lineage diversity, morphological and genetic divergence in *Daphnia magna* (Crustacea) among Chinese lakes at different altitudes. Contributions to Zoology (Amsterdam, Netherlands) 89(4): 450–470. https://doi.org/10.1163/18759866-bja10011

Mahecha MD, Bastos A, Bohn FJ, Eisenhauer N, Feilhauer H, Hartmann H, Hickler T, Kalesse-Los H, Migliavacca M, Otto FEL, Peng J, Quaas J, Tegen I, Weigelt A, Wendisch M, Wirth C (2022) Biodiversity loss and climate extremes—Study the feedbacks. Nature 612(7938): 30–32. https://doi.org/10.1038/d41586-022-04152-y

Martínez A, Bonaglia S, Di Domenico M, Fonseca G, Ingels J, Jörger KM, Laumer C, Leasi F, Zeppilli D, Baldrighi E, Bik H, Cepeda D, Curini-Galletti M, Cutter AD, dos Santos G, Fattorini S, Frisch D, Gollner S, Jondelius U, Kerbi A, Kocot KM, Majdi N, Mammola S, Martín-Durán JM, Menegotto A, Montagna PA, Nascimento FJA, Puillandre N, Rognant A, Sánchez N, Santos IR, Schmidt-Rhaesa A, Schratzberger M, Semprucci F, Shimabukuro M, Sommerfield PJ, Struck TH, Sørensen MV, Wallberg A, Worsaae K, Yamasaki H, Fontaneto D (2025) Fundamental questions in meiofauna research highlight how small but ubiquitous animals can improve our understanding of Nature. Communications Biology 8(1): 449. https://doi.org/10.1038/s42003-025-07888-1

Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach AL, Smith VH, Staley JT (2006) Microbial biogeography: Putting microorganisms on the map. Nature Reviews. Microbiology 4(2): 102–112. https://doi.org/10.1038/nrmicro1341

Mi X, Feng G, Hu Y, Zhang J, Chen L, Corlett RT, Hughes AC, Pimm S, Schmid B, Shi S, Svenning JC, Ma K (2021) The global significance of biodiversity science in China: An overview. National Science Review 8: nwab032. https://doi.org/10.1093/nsr/nwab032

Montero-Pau J, Gómez A, Muñoz J (2008) Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. Limnology and Oceanography: Methods 6(6): 218–222. https://doi.org/10.4319/lom.2008.6.218

Moreno E, Pérez-Martínez C, Conde-Porcuna JM (2016) Dispersal of zooplankton dormant propagules by wind and rain in two aquatic systems. Limnetica 35: 323–336. https://doi.org/10.23818/limn.35.26

Moreno E, Pérez-Martínez C, Conde-Porcuna JM (2019) Dispersal of rotifers and cladocerans by waterbirds: Seasonal changes and hatching success. Hydrobiologia 834(1): 145–162. https://doi.org/10.1007/s10750-019-3919-6

Nowell RW, Almeida P, Wilson CG, Smith TP, Fontaneto D, Crisp A, Micklem G, Tunnacliffe A, Boschetti C, Barraclough TG (2018) Comparative genomics of bdelloid rotifers: Insights from desiccating and nondesiccating species. PLOS Biology 16(4): e2004830. https://doi.org/10.1371/journal.pbio.2004830

Obertegger U, Fontaneto D, Flaim G (2012) Using DNA taxonomy to investigate the ecological determinants of plankton diversity: Explaining the occurrence of *Synchaeta* spp. (Rotifera, Monogononta) in mountain lakes. Freshwater Biology 57(8): 1545–1553. https://doi.org/10.1111/j.1365-2427.2012.02815.x

Örstan A (2018) Taxonomic morphology of the genus *Adineta* (Rotifera: Bdelloidea: Adinetidae) with a new species from a suburban garden. Zootaxa 4524(2): 187–199. https://doi.org/10.11646/zootaxa.4524.2.3

Ortells R, Gómez A, Serra M (2003) Coexistence of cryptic rotifer species: Ecological and genetic characterisation of *Brachionus plicatilis*. Freshwater Biology 48(12): 2194–2202. https://doi.org/10.1046/j.1365-2427.2003.01159.x

Otto SP (2018) Adaptation, speciation and extinction in the Anthropocene. Proceedings, Biological Sciences 285(1891): 20182047. https://doi.org/10.1098/rspb.2018.2047

Partemi R, Debortoli N, Martínez A, Kamburska L, Souffreau C, Matheve H, Vantieghem P, De Meester L, Van Doninck K, Merckx T, Fontaneto D (2024) Weak effect of urbanization on bdelloid rotifers living in lichens. Royal Society Open Science 11(4): 231978. https://doi.org/10.1098/rsos.231978

Peters U, Koste W, Westheide W (1993) A quantitative method to extract moss-dwelling rotifers. Hydrobiologia 255(1): 339–341. https://doi.org/10.1007/BF00025857

Poureskandarian MA, Pourjam E, Pedram M (2023) New evidence on cryptic speciation in soil-inhabiting nematodes, with morphological and molecular characterisation of *Xiphinema sangesarense* n. sp. (Dorylaimida: Longidoridae) from north-central mountains of Iran. Nematology 25(5): 549–563. https://doi.org/10.1163/15685411-bja10238

Puillandre N, Lambert A, Brouillet S, Achaz GJME (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology 21(8): 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Puillandre N, Brouillet S, Achaz G (2021) ASAP: Assemble species by automatic partitioning. Molecular Ecology Resources 21(2): 609–620. https://doi.org/10.1111/1755-0998.13281

Raymond JA (2025) A horizontally transferred bacterial gene aids the freezing tolerance of Antarctic bdelloid rotifers. Proceedings of the National Academy of Sciences of the United States of America 122(10): e2421910122. https://doi.org/10.1073/pnas.2421910122

Ricci C (2017) Bdelloid rotifers: 'sleeping beauties' and 'evolutionary scandals', but not only. Hydrobiologia 796(1): 277–285. https://doi.org/10.1007/s10750-016-2919-z

Ricci C, Fontaneto D (2009) The importance of being a bdelloid: Ecological and evolutionary consequences of dormancy. The Italian Journal of Zoology 76(3): 240–249. https://doi.org/10.1080/11250000902773484

Ricci C, Melone G (2000) Key to the identification of the genera of bdelloid rotifers. Hydrobiologia 418(1): 73–80. https://doi.org/10.1023/A:1003840216827

Rivas Jr JA, Schröder T, Gill TE, Wallace RL, Walsh EJ (2019) Anemochory of diapausing stages of microinvertebrates in North American drylands. Freshwater Biology 64(7): 1303–1314. https://doi.org/10.1111/fwb.13306

Robeson MS, King AJ, Freeman KR, Birky Jr CW, Martin AP, Schmidt SK (2011) Soil rotifer communities are extremely diverse globally but spatially autocorrelated locally. Proceedings of the National Academy of Sciences of the United States of America 108(11): 4406–4410. https://doi.org/10.1073/pnas.1012678108

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34(12): 3299–3302. https://doi.org/10.1093/molbev/msx248

Santamaria CA (2019) Molecular taxonomy of endemic coastal *Ligia* isopods from the Hawaiian Islands: Re-description of *L. hawaiensis* and description of seven novel cryptic species. PeerJ 7: e7531. https://doi.org/10.7717/peerj.7531

Segers H (2007) Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. Zootaxa 1564(1): 1–104. https://doi.org/10.11646/zootaxa.1564.1.1

Segers H, Shiel RJ (2008) Diversity of cryptic Metazoa in Australian freshwaters: A new genus and two new species of sessile rotifer (Rotifera, Monogononta, Gnesiotrocha, Flosculariidae). Zootaxa 1750(1): 19–31. https://doi.org/10.11646/zootaxa.1750.1.2

Seto KC, Fragkias M, Güneralp B, Reilly MK (2011) A meta-analysis of global urban land expansion. PLOS ONE 6(8): e23777. https://doi.org/10.1371/journal.pone.0023777

Shain DH, Iakovenko NS, Cridge AG, Novis PM, Plášek V, Dearden PK (2022) Microinvertebrate colonization of New Zealand's thermally extreme environments. Evolutionary Biology 49(4): 414–423. https://doi.org/10.1007/s11692-022-09578-w

Shain DH, Rogozhina I, Fontaneto D, Nesje A, Saglam N, Bartlett J, Zawierucha K, Kielland ØN, Dunshea G, Arnason E, Rosvold J (2024) Ice-inhabiting species of Bdelloidea Rotifera reveal a pre-Quaternary ancestry in the Arctic cryosphere. Biology Letters 20(6): 20230546. https://doi.org/10.1098/rsbl.2023.0546

Shmakova L, Malavin S, Iakovenko N, Vishnivetskaya T, Shain D, Plewka M, Rivkina E (2021) A living bdelloid rotifer from 24,000-year-old Arctic permafrost. Current Biology: CB 31(11): R712–R713. https://doi.org/10.1016/j.cub.2021.04.077

Surmacz B, Vecchi M, Fontaneto D, Budzik K, Godziek J, Matsko Y, Stec D (2025) COI metabarcoding with a curated reference database and optimized protocol provides a reliable species-level diversity assessment of tardigrades. Integrative Zoology: 1–16. https://doi.org/10.1111/1749-4877.12972

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22): 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Uchida K, Matanle P, Li Y, Fujita T, Hiraiwa MK (2025) Biodiversity change under human depopulation in Japan. Nature Sustainability 8(8): 883–893. https://doi.org/10.1038/s41893-025-01578-w

Walczyńska A, Fontaneto D, Kordbacheh A, Hamil S, Jimenez-Santos MA, Paraskevopoulou S, Pociecha A, Zhang W (2024) Niche differentiation in rotifer cryptic species complexes: A review of environmental effects. Hydrobiologia 851(12–13): 2909–2926. https://doi.org/10.1007/s10750-023-05291-7

Wang XL, Xiang XL, Xia MN, Han Y, Huang L, Xi YL (2014) Differences in life history characteristics between two sibling species in *Brachionus calyciflorus* complex from tropical shallow lakes. Annales de Limnologie - International. Annales de Limnologie 50(4): 289–298. https://doi.org/10.1051/limn/2014024

Wang Q, Li Y, Wang W, Yang Y (2021) Species diversity and phylogenetic analysis of bdelloid rotifers in Guangdong Province. China Environmental Science 41: 4367–4377. https://doi.org/10.3969/j.issn.1000-6923.2021.09.044

Wang L, Deng Z, Blair D, Hu W, Yin M (2023a) Phylogeography and genetic diversity of the *Scapholeberis kingii* species complex (Cladocera: Daphniidae) in China. Molecular Phylogenetics and Evolution 181: 107725. https://doi.org/10.1016/j.ympev.2023.107725

Wang W, Yang Y, Cui Z, Chen M, Ma X, Wang Q (2023b) High diversity and strong habitat preference of bdelloid rotifers in the moss and leaf litter from a small area of urban plain and adjacent hill in China. Biodiversity and Conservation 32(8–9): 2769–2789. https://doi.org/10.1007/s10531-023-02630-x

Wang W, Yang Y, Örstan A, He Z, Wang Q (2025) Species diversity of bdelloid rotifer (Rotifera, Bdelloidea) in different areas in China, with a description of two new species. ZooKeys 1229: 1–23. https://doi.org/10.3897/zookeys.1229.113385

Wilson CG, Sherman PW (2010) Anciently asexual bdelloid rotifers escape lethal fungal parasites by drying up and blowing away. Science 327(5965): 574–576. https://doi.org/10.1126/science.1179252

Wilson CG, Sherman PW (2013) Spatial and temporal escape from fungal parasitism in natural communities of anciently asexual bdelloid rotifers. Proceedings, Biological Sciences 280(1765): 20131255. https://doi.org/10.1098/rspb.2013.1255

Wilson CG, Pieszko T, Nowell RW, Barraclough TG (2024) Recombination in bdelloid rotifer genomes: Asexuality, transfer and stress. Trends in Genetics: TIG 40(5): 422–436. https://doi.org/10.1016/j.tig.2024.02.001

Woolfit M, Bromham L (2005) Population size and molecular evolution on islands. Proceedings, Biological Sciences 272(1578): 2277–2282. https://doi.org/10.1098/rspb.2005.3217

Wu D, Du E, Eisenhauer N, Mathieu J, Chu C (2025) Global engineering effects of soil invertebrates on ecosystem functions. Nature 640(8057): 120–129. https://doi.org/10.1038/s41586-025-08594-y

Xiang XL, Xi YL, Wen XL, Zhang GEN, Wang JX, Hu KE (2011) Genetic differentiation and phylogeographical structure of the *Brachionus calyciflorus* complex in eastern China. Molecular Ecology 20(14): 3027–3044. https://doi.org/10.1111/j.1365-294X.2011.05147.x

Xiang XL, Jiang R, Tao Y, Chen Y, Xi YL (2016a) Differences in life history characteristics among three sympatric evolutionary species of the *Rotaria rotatoria* complex. Journal of Freshwater Ecology 31(3): 351–360. https://doi.org/10.1080/02705060.2016.1141379

Xiang XL, Chen YY, Han Y, Wang XL, Xi YL (2016b) Comparative studies on the life history characteristics of two *Brachionus calyciflorus* strains belonging to the same cryptic species. Biochemical Systematics and Ecology 69: 138–144. https://doi.org/10.1016/j.bse.2016.09.003

Xiang C, Gao F, Jakovlić I, Lei H, Hu Y, Zhang H, Zou H, Wang G, Zhang D (2023) Using PhyloSuite for molecular phylogeny and tree-based analyses. iMeta 2(1): e87. https://doi.org/10.1002/imt2.87

Xiang XL, Wang C, Huang L, Zhu L, Xu J, Wen X, Xi YL (2024) The resistance to short-term dehydration in the bdelloid rotifer *Rotaria rotatoria* from different climate regions. Hydrobiologia 851(12–13): 3185–3198. https://doi.org/10.1007/s10750-023-05375-4

Yang W, Deng Z, Blair D, Hu W, Yin M (2022) Phylogeography of the freshwater rotifer *Brachionus calyciflorus* species complex in China. Hydrobiologia 849(12): 2813–2829. https://doi.org/10.1007/s10750-022-04897-7

Yu D, Wang J, Hu L, Han G, Wang W, Li X, Huang X, Sun Y, Yu S, Tan Y, Su Y, Zhu Y, Xu J, Zhu X, Ma L, Liu C, Dong Y (2025) Habitat complexity and substrate age are primary factors influencing temperate intertidal community assembly. Diversity & Distributions 31(3): e70017. https://doi.org/10.1111/ddi.70017

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics (Oxford, England) 29(22): 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2019) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

Zhu L, Huang R, Feng S, Gao F, Li M, Lian H, Li Z, Cheng X, Yin M, Zhang W, Xi Y, Xiang X (2025) Everything is everywhere, but everywhere is different: Evidence from the phylogeography of asexual bdelloid rotifer *Rotaria rotatoria* species complex. Hydrobiologia 853: 739–762. https://doi.org/10.1007/s10750-025-05963-6

Supplementary material 1

Supplementary figures S1, S2 and tables S1–S6

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